Propanoic acid, 2-hydroxy-, compd. with 3-[2-(dimethylamino)ethyl] 1-(2-ethylhexyl) (4-methyl-1,3-phenylene)bis[carbamate] (1:1)
CAS No. 68227-46-3

U. S. EPA HPV Challenge Program Submission

February 2003
Revised on June 30, 2003
Revised on December 17, 2004

Submitted by

PPG Industries, Inc.
One PPG Place
Pittsburgh, PA 15272

TEST PLAN
Propanoic acid, 2-hydroxy-, compd. with 3-[2-(dimethylamino)ethyl] 1-(2-ethylhexyl) (4-methyl-1,3-phenylene)bis[carbamate] (1:1)
CAS No. 68227-46-3

ENDPOINT	Information Available (Yes/No)	Testing Required (Yes/No)
Physical-chemical Data		
Melting Point	No	No
Boiling Point	No	No
Vapor Pressure	Yes*	No
Water Solubility	Yes	No
Partition Coefficient	Yes*	No
Environmental Fate and Pathway		
Photodegradation	Yes*	No
Stability in Water	Yes*	No
Transport/distribution (Fugacity)	Yes*	No
Biodegradation	Yes	No
Ecotoxicity		
Acute Toxicity to Fish	No	No
Acute Toxicity to Aquatic Invertebrates	Yes	No
Acute Toxicity to Aquatic Plants	Yes	No
Chronic Toxicity to Aquatic Invertebrates	Yes	No
Toxicological Data		
Acute Toxicity	Yes	No
Repeated Dose Toxicity	Yes	No
Repro/Developmental Toxicity	Yes	No
Genetic Toxicity *in vitro* (Gene Mutation)	Yes	No
Genetic Toxicity *in vitro* (Chromosomal Aberration)	Yes	No

*Calculated Data was used.

TABLE OF CONTENTS

1. Sponsoring Company

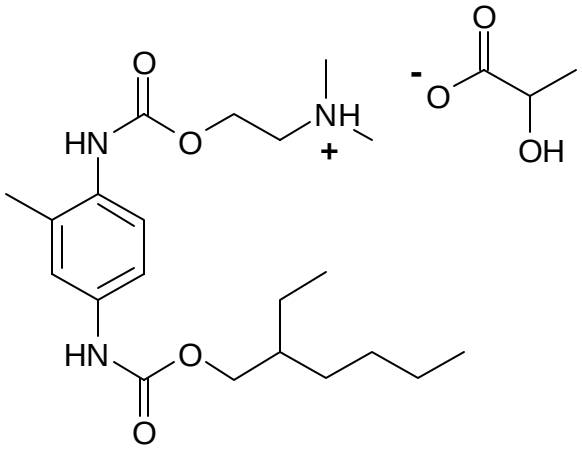
PPG Industries, Inc. is the manufacturer of Propanoic acid, 2-hydroxy-, compd. with 3-[2-(dimethylamino)ethyl] 1-(2-ethylhexyl) (4-methyl-1,3-phenylene)bis[carbamate] (1:1) and is the sponsor of this substance for the U.S. Environmental Protection Agency's HPV Chemical Challenge Program.

2. Test substance

Propanoic acid, 2-hydroxy-, compd. with 3-[2-(dimethylamino)ethyl] 1-(2-ethylhexyl) (4-methyl-1,3-phenylene)bis[carbamate] (1:1) is an isolated intermediate and subsequently used to produce a resin that is the component of paint products. This test substance is manufactured at one PPG production facility. The majority of this test substance (~70 to 85%) is currently used at that PPG location. Filled stainless steel semi-bulk tanks containing the test substance, as an isolated intermediate, are transported and stored at a warehouse for captive use on-site. The remaining small portion of the test substance is sold to two other companies, where it is also used as an intermediate that undergoes further reaction to form a resin. Filled lined steel drums are shipped via common carrier to the customers.

Since the test substance is an intermediate to be used for a production of resin and the vapor pressure is expected to be very low, the only reasonably anticipated potential exposure would be by skin contact and would occur in an occupational setting to a limited number of workers at PPG and customer sites. There would be no reasonably anticipated exposure to the general public.

The molecular structure of the test substance is as follows:



The test substance is produced commercially as 75% solids [Propanoic acid, 2-hydroxy-, compd. with 3-[2-(dimethylamino)ethyl] 1-(2-ethylhexyl) (4-methyl-1,3-phenylene)bis[carbamate] (1:1)] in the presence of Methyl Isobutyl Ketone (MIBK) solvent (2-3%), 2-butoxy ethanol (6-7%),

and water (15-17%). At this concentration in this solvent system, the test substance is a clear, light yellow, liquid. In order to prepare an isolated sample of the test substance (100% of CAS No 68227-46-3) for HPV testing, an attempt was made to drive off the solvents by distillation. However, this removal of excess solvents from the substance resulted in the formation of polymeric by-products. Therefore, it was not possible to prepare the isolated (solvent-free) test substance for HPV testing. Instead, the test substance was prepared in MIBK as usual and then diluted with water. The aqueous solution was then vacuum stripped to remove the MIBK and a portion of the water, resulting in a sample containing the test substance at 71% solids in water.

3. Criteria for Determining Adequacy of Data

All relevant data were reviewed and assessed for adequacy according to the standards of Klimisch *et al*. (1977). Four reliability categories, 1-reliable without restriction, 2-reliable with restriction, 3-not reliable, and 4-not assignable, have been established and a rating of 1 and 2 were considered to be adequate.

4. Test Plan

4.1 Physical/Chemical Properties

No measured data are available for melting point, boiling point, vapor pressure, octanol/water and partition coefficient. Because producing pure material (free of solvents) for the purposes of determining a melting point and a boiling point is not possible, no meaningful data can be generated. Therefore, no testing for melting and boiling was conducted.

Data for vapor pressure and partition coefficient (Kow) are estimated (calculated) using a modeled approach. The vapor pressure is estimated to be 2.62E-8 mm Hg and the partition coefficient (Log KOW) is estimated to be 4.38. Measurement of water solubility was conducted (Project Report 23779). The test substance was very soluble in water and the water solubility was determined to be 281 g/l at 20 °C.

4.2 Environmental Fate/Pathways

Data for photodegradation, stability in water, and environmental transport were estimated using the EPIWIN/HYDROWIN/AOPWIN program. The estimated photodegradation hydroxyl radical rate constant was estimated to be 113.7966 E-12 cm^3/molecule-sec with a half-life calculated to be 1.128 hours. Aqueous base/acid-catalyzed hydrolysis indicates that the estimated total Kb for pH >8 is 1.343 E+1 L/mol-sec with a half-life calculated to be 14.339 hours. Level III fugacity modeling indicates that the test substance should partition to water (14.4 %), air (3.23E-5 %), soil (79.1 %), and sediment (6.52 %). The data indicated that the test substance is not readily biodegradable (Project Report 23693).

4.3 Ecotoxicity

In its guidance on data development for the HPV program, EPA recommends use of chronic toxicity testing in *Daphnia* (in place of acute toxicity testing in fish and *Daphnia*) and toxicity to plant (algae) for chemicals determined to have a Log Kow equal to or greater than 4.2. However, the EPA guidance further indicates that acute aquatic toxicity testing may be relevant for chemicals that are dispersible in water.

Since the estimated Log Kow value of this test substance is greater than 4.2 and the material would be somewhat dispersible, PPG conducted the acute toxicity to *Daphnia* (Project Report 24139) in addition to chronic toxicity to *Daphnia* (Project Report 24048) and acute toxicity to algae (Project Report 23966). Based on the data obtained from the acute toxicity to *Daphnia* and algae, the 48-hour EC_{50} value for *Daphnia magna* was 4.2 mg/L and the 96-hour EC_{50} value for algae was 0.07 mg/L for area under the growth curve and 0.16 mg/L for growth rate. In a chronic toxicity to *Daphnia magna*, the Day 7 EC_{50} to *Daphnia* reproduction was estimated as 0.69 mg/L. The Day 21 No Observed Effect Concentration (NOEC) to *Daphnia* reproduction was 0.365 mg/L. As all parental *Daphnia* were dead at the 1.369 and 4.653 mg/L concentrations and no reproduction effect was noted at 0.365 mg/L at Days 14 and 21, the EC_{50} for these time points was estimated to be between 0.365 mg/L to 0.69 mg/L (between the NOEC and Day 7 EC_{50}).

4.4 Human Health Data

4.4.1 Acute Mammalian Toxicity

There are no existing data on acute mammalian toxicity for this material. In order to minimize the number of animals needed to estimate the health effects endpoints required to fulfill HPV toxicity testing requirements, a testing strategy was conducted to incorporate one acute toxicity study (OECD Guideline 425, oral toxicity, up and down method, Project Report 23655) with a supplemental component of *in-vitro* cytotoxicity test (Project Report 3507). The *in-vitro* cytotoxicity test (Neutral Red Uptake Bioassay) was conducted to estimate a starting dose level for the *in-vivo* acute oral toxicity study. This testing approach was intended to minimize the number of animals used for testing to develop the needed information. The Neutral Red Uptake Bioassay is used to quantitatively measure the toxicity of a test substance to Balb/c 3T3 cultures by comparing the neutral red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochloride) uptake of test substance treated Balb/c 3T3 cultures. The estimated LD_{50} of the test substance from the Neutral Red Uptake Bioassay was determined to be 489 mg/kg. Based on this result, the starting dose level for acute oral toxicity study was set as 175 mg/kg.

In the acute oral toxicity study (utilizing the up and down method), fifteen females received test substance at 175, 550 or 2000 mg/kg. Five out of 9 animals treated at 2000 mg/kg died prematurely on Days 2 and 3. Predominant clinical signs included piloerection, cold to touch, laboured breathing, staggering, subdued behaviour, tremors and hunched appearance. At 2000 mg/kg, 2 of the 4 surviving animals had lost up to 25% of their Day 1 body weights by Day 15. Under the conditions of the study, the median lethal oral dose (LD_{50}) for the test substance in Sprague-Dawley rats was estimated to be equal to 2000 mg/kg.

In summary, the estimated LD_{50} value from the *in-vitro* cytotoxicity study that was used to set the starting dose for the acute oral toxicity study was significantly different than the actual LD_{50} value determined in the *in-vivo* oral study. In addition, the LD_{50} value from the acute oral study was determined to be exactly at 2000 mg/kg. Therefore, due to the dosing regimen required in the up and down method, a greater than expected number of animals was needed in the acute oral toxicity study.

4.4.2 Repeated Dose Mammalian Toxicity

In order to fulfill HPV toxicity testing requirements, the June 2003 revision of the Test Plan stated that a combined repeated dose/reproductive/developmental toxicity study (OECD Guideline 422) was planned in order to fulfill the repeated dose, reproductive, and developmental toxicity endpoints while minimizing the number of animals required. However, unexpected steep dose responses were noted in the one-week range-finding study and it was very difficult to set the doses for the pregnant animals based on the data obtained from the range-finding study. Therefore, a 4-week repeated dose toxicity study followed by a reproductive/developmental toxicity study was conducted.

In the 28-Day repeated dose toxicity study (OECD Guideline 407, Project Report 24231), three groups of 5 male and 5 female (Low, Intermediate and High dose groups) Sprague-Dawley rats were dosed daily for 4 consecutive weeks by gavage at levels of 2, 7.5 and 30 mg/kg/day. A further group of 5 male and 5 female rats received vehicle (water for irrigation) only and acted as a Control group. There were overall body weight and food consumption reduction at 7.5 mg/kg/day and body weight and food consumption reduction during the first week of treatment at 30 mg/kg/day. Isolated incidences of excess salivation were noted throughout the study in a number of animals treated at 30 mg/kg/day. There was some evidence of an effect on testes (bilateral tubular atrophy) and epididymides (sloughing of spermatogenic cells) of animals given 30 mg/kg/day. The No Adverse Effect Level (NOAEL) for both sexes was considered to be 2 mg/kg/day.

4.4.3 Genetic Toxicity

In order to fulfill HPV toxicity testing requirements while minimizing the number of animals needed to estimate the health effects endpoints, PPG conducted two *in-vitro* genetic toxicity studies (OECD Guidelines 471 and 473). In a mutagenicity study in S. *typhimurium* strains TA98, TA100, TA1535, and TA1537 and E. coli strain WP2uvrA in the absence and presence of a metabolic activation system (Project Report 23769), the test substance produced negative results. The test substance did not induce structural aberrations when tested with Chinese hamster ovary cells *in vitro* (Project Report 23963). However, slight increases in polyploidy were noted in the cultures in the absence of S9 mix, 22 h treatment, 48 h harvest and in the presence of S9 mix, 6 h treatment, 24 h harvest. These increases are not likely to be biologically relevant based on the small increases in the number of polyploid cells at or near toxic concentrations and no dose response curve being evident.

4.4.4 Reproductive/Developmental Toxicity

As described in Section 4.4.2, the June 2003 revision of the Test Plan proposed to conduct a combined repeated dose/reproductive/developmental toxicity study (OECD Guideline 422) in order to fulfill the repeated dose, reproductive, and developmental toxicity endpoints while minimizing the number of animals required. However, unexpected steep dose responses were noted in the one-week range-finding study and it was very difficult to set the doses for the pregnant animals based on the data obtained from the range-finding study. Therefore, a 4-week repeated dose toxicity study followed by a reproductive/developmental toxicity study was conducted.

In the reproductive/developmental toxicity screening study (OECD Guideline 421, Project Report 24474), Sprague-Dawley rats were randomised into 3 test groups and one negative Control group, each containing 10 males and 10 females. Males were treated once daily for 2 weeks prior to mating through until necropsy after 4 weeks of treatment. Females were treated once daily for 2 weeks prior to mating, then throughout mating, gestation and until *ca* Day 4 of lactation. Dose levels were 0, 2, 7.5 and 50 mg/kg/day. Toxicity of test substance was indicated at 7.5 and 50 mg/kg/day by decreases in body weight gain and food consumption, and at 50 mg/kg/day also by piloerection and salivation. At 50 mg/kg/day, 6/10 males had low testes weights; these testes had marked seminiferous epithelial degeneration and mild interstitial cell hyperplasia. There was oligospermia and sloughing of spermatogenic cells in the epididymides of these animals; group mean epididymides weight at this level was lower than Control. At 50 mg/kg/day, 2/10 pairs failed to mate; both males had low testes weights. The mean duration of gestation at 50 mg/kg/day was slightly increased. The mean number of implants at 50 mg/kg/day was lower than Control, and pup mortality markedly increased, such that surviving pups at this level were sacrificed for welfare considerations. At 7.5 mg/kg/day, reproductive effects were confined to a reduced number of implants, and therefore of pups born, and slight reductions in mean litter and pup weights. The No Observed Effect Level (NOEL) for both sexes of adults, and for reproductive effects, was 2 mg/kg/day.

5. References

(1) A. Hogg (2004) Inveresk Research Unpublished Project Report 23779.
(2) T. Hargreaves (2004) Inveresk Research Unpublished Project Report 23693.
(3) T. Hargreaves (2004) Inveresk Research Unpublished Project Report 24139.
(4) T. Hargreaves (2004) Inveresk Research Unpublished Project Report 23966.
(5) T. Hargreaves (2004) Inveresk Research Unpublished Project Report 24048.
(6) H. Raabe (2003) Institute for In Vitro Sciences Unpublished Project Report 3507.
(7) A. Hutchinson (2004) Inveresk Research Unpublished Project Report 23655.
(8) G. Morgan (2004) Inveresk Research Unpublished Project Report 24231.
(9) F. M. Stevenson (2004) Inveresk Research Unpublished Project Report 23769.
(10) E. Murie (2004) Inveresk Research Unpublished Project Report 23963.
(11) S. Barton (2004) Inveresk Research Unpublished Project Report 24474.

`